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First COVID-19 case in Zambia — Comparative phylogenomic analyses of SARS-CoV-2 detected in African countries

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ABSTRACT

Since its first discovery in December 2019 in Wuhan, China, COVID-19, caused by the novel coronavirus SARS-CoV-2, has spread rapidly worldwide. While African countries were relatively spared initially, the initial low incidence of COVID-19 cases was not sustained for long due to continuing travel links between China, Europe and Africa. In preparation, Zambia had applied a multisectoral national epidemic disease surveillance and response system resulting in the identification of the first case within 48 h of the individual entering the country by air travel from a trip to France. Contact tracing showed that SARS-CoV-2 infection was contained within the patient's household, with no further spread to attending health care workers or community members. Phylogenomic analysis of the patient's SARS-CoV-2 strain showed that it belonged to lineage B.1.1., sharing the last common ancestor with SARS-CoV-2 strains recovered from South Africa. At the African continental level, our analysis showed that B.1 and B.1.1 lineages appear to be predominant in Africa. Whole genome sequence analysis should be part of all surveillance and case

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Introduction

The WHO declared COVID-19, caused by SARS-CoV-2, a Public Health Emergency of International Concern (PHEIC) on 30 January 2020 and later a pandemic on 11 March 2020 (WHO, 2020a). As of 28 September 2020, there were 32.7 million COVID-19 cases with 991,000 deaths worldwide reported to the WHO (2020b). All African countries have been affected and have reported a total of 1,172,342 COVID-19 cases, including 25,481 deaths (WHO, 2020b). As the global COVID-19 events unfolded, and Africa's first COVID-19 case was reported from Egypt on 14 February 2020, many African countries prepared for the arrival of COVID-19 (Kapata et al., 2020). Zambia embarked on the intensification of the disease surveillance and emergency preparedness and response systems, including activating the Public Health Emergency Operations Centre (PHEOC). As part of preparedness activities, ports of entry were put on alert and thermal scanners were installed for screening incoming passengers at airports and ground crossing. Key to the preparedness was ensuring that local and international networks were functional, including staff training and knowledge exchange, such as among the Pan-African Network For Rapid Research, Response, Relief and Preparedness for Infectious Disease Epidemics (PANDORA-ID-NET) consortia members, WHO and African Union Member States. Isolation facilities were identified and the incident management system (IMS) was activated at various levels. Ports of entry health staff were crucial for early detection of imported cases given that Zambia was at risk for importation of COVID-19 (Gilbert et al., 2020). The University Teaching Hospital (UTH) Virology Biosafety Level-2 (BSL-2) Laboratory, and the University of Zambia School of Veterinary Medicine BSL-3 Laboratory in Lusaka, Zambia were identified as national COVID-19 diagnostic testing centres and for molecular analyses of SARS-CoV-2 lineages. We report the identification and clinical management of the first COVID-19 case from Zambia, and present the phylogenetic analyses of the patient's SARS-CoV-2 isolate, comparing it with other SARS-CoV-2 lineages reported from other African countries.

Methods

Ethical review and approval to publish

Ethical approval for case study and phylogenomic sequencing, and publication of this case study was obtained from the University of Zambia Biomedical Research Ethics Committee (REF. NO. 002-07-20) and the National Health Research Authority.

Case identification, clinical features and epidemiological investigation

As of 1 February 2020, all travellers arriving at Lusaka Kenneth Kaunda International Airport in Lusaka, Zambia are screened for fever in line with the Zambian Ministry of Health COVID-19 public health response guidelines. On 15 March 2020, a 39-year-old man, accompanied by his wife and two children, returned to Zambia from a 2-week trip to Europe (France), the family was placed under self-quarantine. On 16 March 2020, nasopharyngeal samples collected by the rapid response team (RRT) for the detection of the presence of SARS-CoV-2 nucleic acid were positive only in the case under study. Further questioning indicated that the patient had a slightly dry and sore throat. On 18 March, 3 days after returning to Lusaka, our case developed a mild fever (38.0 °C) and was treated using paracetamol 1 gm three times a day orally for 5 days. Similar to reports from Europe and the USA (Spinato et al., 2020; Yan et al., 2020a; Yan et al., 2020b), patient had anosmia and complained of a metallic taste in the mouth 2 days prior to complete loss of taste. Our patient did not have any co-morbidities and was placed under quarantine for 21 days after testing positive to SARS-CoV-2. On day-5 (20th March 2020), he developed a mild cough, persistent fever (>38 °C), chest discomfort and clinical examination revealed bilateral chest crepitations. There were bilateral infiltrates on chest X-ray and full blood count showed mild lymphopenia (Table 1; Figure 1). He was thus classified as having 'moderate COVID-19 pneumonia' and transferred for further clinical management at the national COVID-19-specific hospital where he was isolated, and was given a course of azithromycin (and then switched to amoxycillin–clavunate acid) and supportive care. Smell and taste abnormalities resolved within 5 days. The patient did not require intensive care and steadily improved with resolution of fever and resolution of infiltrates on repeat chest radiograph. He made a full recovery by day 21. Repeated contact tracing within the household, showed his wife testing positive for SARS-CoV-2, but his children remained negative. There was no further spread to other family members or attending health care workers.

SARS-CoV-2 detection and molecular sequencing

Specimen collection and RNA extraction

Nasopharyngeal and oropharyngeal swab specimens were collected on 16 March 2020 in accordance with the CDC recommendations (CDC, 2020), and samples were processed using

Table 1
Basic metabolic and complete blood count laboratory results.

Test	Results on 21 March 2020	Results on 22 March 2020	Result on 29 March 2020
WBC	3.7×10^9/L		5.5×10^9/L
RBC	4.24×10^{12}/L		4.46×10^{12}/L
Hb	12.5 g/dL		12.9 g/dL
MCV	93 fl		94 fl
Hct	39.3%		41.9%
PLT	164×10^9/L		678×10^9/L
NEUT	48%		61.9%
LYMPOCYTES	42.1%		32.5%
MONOCYTES	9.9%		5.6%
CREATININE		75.0 μmol/L	84.9 μmol/L
AST		24.4 u/L	29.9 u/L

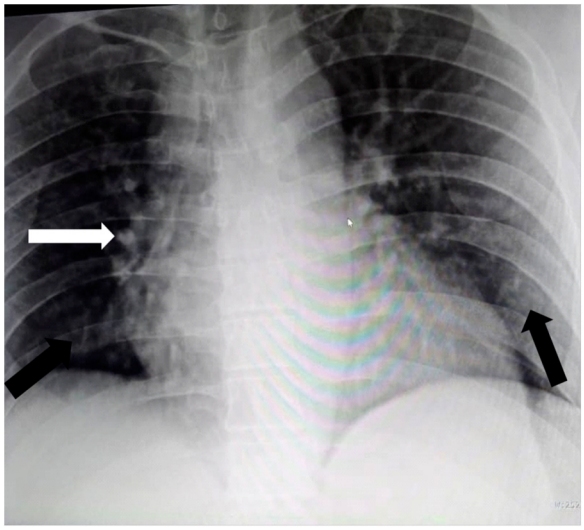

Figure 1. Chest X-ray (CXR) of the first case of COVID-19 in Zambia. The CXR was taken on day 4 after the onset of illness. White arrow shows nodular infiltrates suspected to be micro-abscesses.
Black arrows show bilateral infiltrates affecting lower zones mostly peripheral and the centre.

standard reverse transcription polymerase chain reaction (RT-PCR) methodology for SARS-CoV-2 detection.

Whole genome sequencing

For whole genome sequencing, the Sanger method was employed using several overlapping primers designed using Geneious software version 10.0.9. The list of primers and their combinations for RT-PCR assays and sequencing are listed in Table S1. The complete genome of SARS-CoV-2 investigated in this study was deposited in GenBank (accession no. MT790522). The whole SARS-CoV-2 genome generated in this study, together with other selected SARS-CoV-2 sequences accessed from GISAID database were aligned using the FFT-NS-2 algorithm available in the multiple sequence alignment programme (MAFFT) using default settings. (Katoh et al., 2019) The selection included mostly representation from all African countries that had deposited whole genomes in the GISAID database as of 30 August 2020. The resulting final alignment was then uploaded to the IQ-TREE webserver (Trifinopoulos et al., 2020) for construction of a maximum likelihood phylogeny using the general time reversible nucleotide substitution model and rate of heterogeneity set to gamma (GTR + G), otherwise default settings. Branch robustness was estimated using ultrafast bootstrapping tool available in IQ-TREE with 1000 replicates (Minh et al., 2013). The ML tree was then rooted using TempEst (version 1.5.1) (Rambaut et al., 2016), which estimated the best-fitting root of this phylogeny using the heuristic residual mean squared function, aimed at minimizing the variance of root-to-tip distances. The resultant ML tree file was edited using iTOL (Letunic and Bork, 2019). Viral lineages were identified in the phylogeny according to the recently described nomenclature (Rambaut et al., 2020) as well as demonstrated recently in Uganda (Bugembe et al., 2020).

Results

Phylogenetic and sequence analysis

Phylogenomic analysis showed that the detected SARS-CoV-2 belonged to lineage B.1.1, sharing the most common recent ancestor with viruses detected in South Africa (Figure 2). At the African continental level, our analysis showed that lineage B.1.1 was detected in many countries in Africa, including Nigeria, Morocco, Gambia, Ghana, Senegal, Kenya and South Africa. Outside Africa, this monophyletic group comprised strains from France, Spain and Argentina. While our phylogenomic tree clearly showed that all the known SARS-CoV-2 lineages are present in Africa, lineages B.1 and B.1.1 were seemingly dominating the continent.

When compared with Wuhan-Hu-1 (accession no. MN908947), the SARS-CoV-2 investigated in this study showed nine nucleotide differences: C241 T, C3037 T, C3796 T, T11725C, C14408 T, A23403 G, G28881A, G28882A and G28883C. Four amino acid differences were observed: P4715 L (orf1ab polyprotein), D614 G (surface glycoprotein), R203 K and G204R in the nucleocapsid phosphoprotein.

Discussion

While it was not possible to establish where our first Zambia COVID-19 patient contracted the infection while traveling in Europe or in transit, phylogenomic analysis placed the Zambian SARS-CoV-2 into lineage B.1.1 (Figure 2), a lineage that has spread to various countries in Europe, South America, Asia, Oceania and Africa (https://github.com/cov-lineages/lineages). The close clustering of the Zambian virus with SARS-CoV-2 sequences from South Africa (Figure 2) may imply a common origin of B.1.1 lineage in southern Africa. Our phylogenomic comparisons of SARS-CoV-2 detected in African countries revealed the presence of all the major lineages that have been found globally. This implies that some African countries may have delayed in banning international flights from other high-risk continents and closing of borders with neighbouring countries. As such, many countries in Africa remained vulnerable to importation of several SARS-CoV-2 lineages, which compounds tracking of virus transmission chains and pandemic control efforts. Limiting multiple introductions of imported cases and prudent management of identified cases at local or regional level through efficient community testing, contact tracing and quarantine measures are key considerations for present and future responses to pandemic threats. Zambia had put in place an alert surveillance system that was able to identify the initial cases very quickly.

The B.1.1 lineage has been defined by 28881GA, 28882GC and 28883GC single-nucleotide polymorphisms (SNPs), but our strain showed some variation by having 28882GA. Analysis of the genome of the Zambia virus revealed only four amino acid differences when compared with Wuhan-Hu-1, which included the D614 G mutation which has been observed to correlate with increased case fatality rates (Becerra-Flores and Cardozo, 2020). The SARS-CoV-2 reported in this study also had the P4715 L mutation which has been observed to occur in almost all strains with D614 G mutation, which might affect the speed of virus replication (Koyama et al., 2020). SARS-CoV-2 variants with P4715 L predominate in Europe and the USA (Koyama et al., 2020). While most (if not all) of the complete genomes of SARS-CoV-2 deposited in public databases were sequenced using next generation sequencing (NGS) platforms, we utilized the Sanger method, which is more widely available in Africa when compared with NGS. As laboratory capacities for NGS are yet to be developed in many African countries, the Sanger method could still be used to sequence a number of complete genomes enough to provide a better understanding of the molecular epidemiology of SARS-CoV-2 in Zambia.

The epidemic preparedness and response in Zambia remains on high alert for the COVID-19 pandemic and capacity for contact tracing in the community has been built steadily over time due to experience with previous outbreaks such as Lujo haemorrhagic

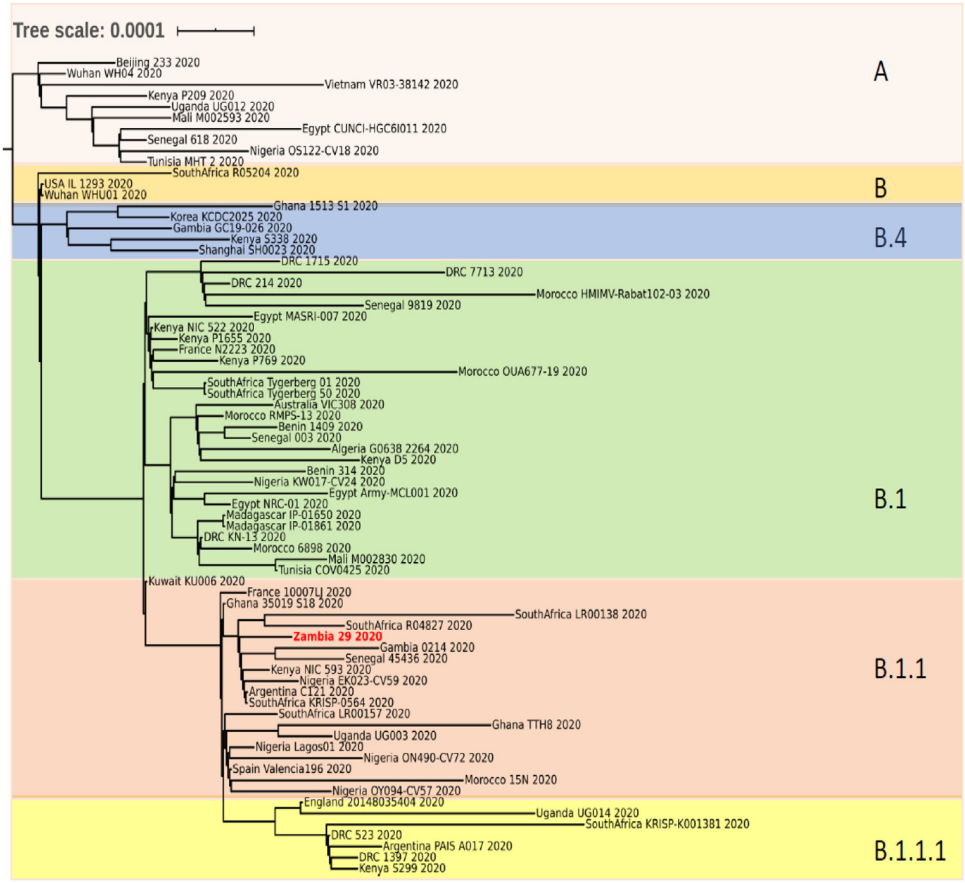

Figure 2. A maximum likelihood tree showing phylogenetic relationships of the complete genome of SARS-CoV-2 detected in a patient in Zambia and other 73 SARS-CoV-2 genomes retrieved from the GISAID database. Of the 73 genomes, 59 were a selection representing African countries that had deposited whole genomes in the GISAID database. The genome generated in this study is shown in red. Viral lineages are shown as shaded areas. The scale shows nucleotide substitutions per site (see attached file for clearer image).

fever and cholera (Paweska et al., 2009; Kapata et al., 2018) as well as ongoing surveillance for influenza and haemorrhagic fever viruses in humans and animals including wildlife (Simulundu et al., 2011; Ogawa et al., 2015; Theo et al., 2012; Changula et al., 2018; Kajihara et al., 2019; Simusika et al., 2020). These activities have contributed to capacity building for the various multi-disciplinary teams working in the health facilities including laboratories for infectious diseases and public health emergencies.. Health system strengthening for future outbreaks using the 'One Health' multisectoral approach has also proved to be a key ingredient to mounting an effective response towards prevention of spread of COVID-19. The COVID-19 outbreak, though originally from China, was detected in Zambia as a result of intercontinental spread largely facilitated by international travel, demonstrating the role of globalization in aiding SARS-CoV-2 transmission across international borders including in Africa (Bugembe et al., 2020). Genotypic analysis of SARS-CoV-2 from all Asia, Europe and Americas shows that the genes encoding the S proteins and RNA polymerase, RNA primase and nucleoprotein, undergo frequent mutations which are important for vaccine development in disease control. Discriminating and comparative analyses of vSARS-CoV-2 isolates can be useful in genetic epidemiology. An understanding of the different genotypes of SARS-CoV-2 strains that are circulating in Zambia and in the Central African region may have implications for vaccine development, transmission and virulence

Conclusion

We report the case history and phylogenomic analyses of SARS-CoV-2 for the first COVID-19 case detected in Zambia. The case history and molecular epidemiology data do not rule out the possibility of direct or indirect importation of the virus from Europe. Phylogenomic analysis showed that the detected SARS-CoV-2 belonged to lineage B.1.1 sharing the most common recent ancestor with SARS-CoV-2 strains recovered from South Africa. At the African continental level, our analysis showed that B.1 and B.1.1 lineages appear to be predominant in Africa. Whole genome sequence analysis should be part of all surveillance activities to monitor the origin and evolution of SARS-CoV-2 lineages across Africa.

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Ethics approval

Ethics approval was obtained from the University of Zambia Biomedical Research Ethics Committee and the National Health Research Authority

Conflict of interest

All authors gave approval and declared no conflict of interest

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